United States Environmental Protection Agency

EPAs ToxCast Research Program: Developing Predictive Bioactivity Signatures for Chemicals

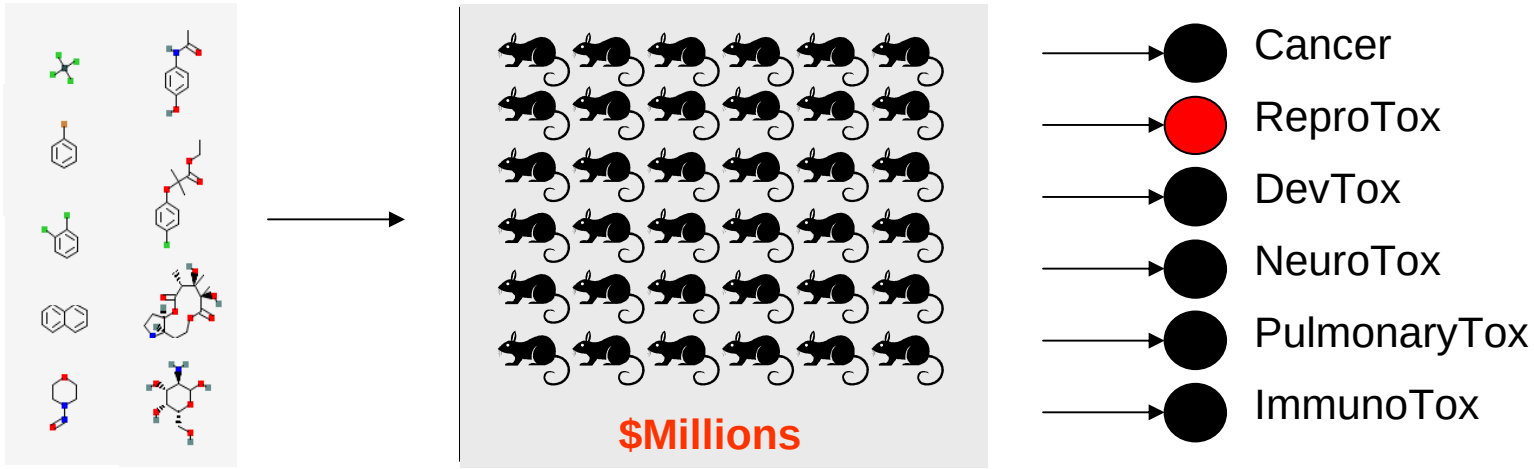
HESI SOT Luncheon
Seattle Washington

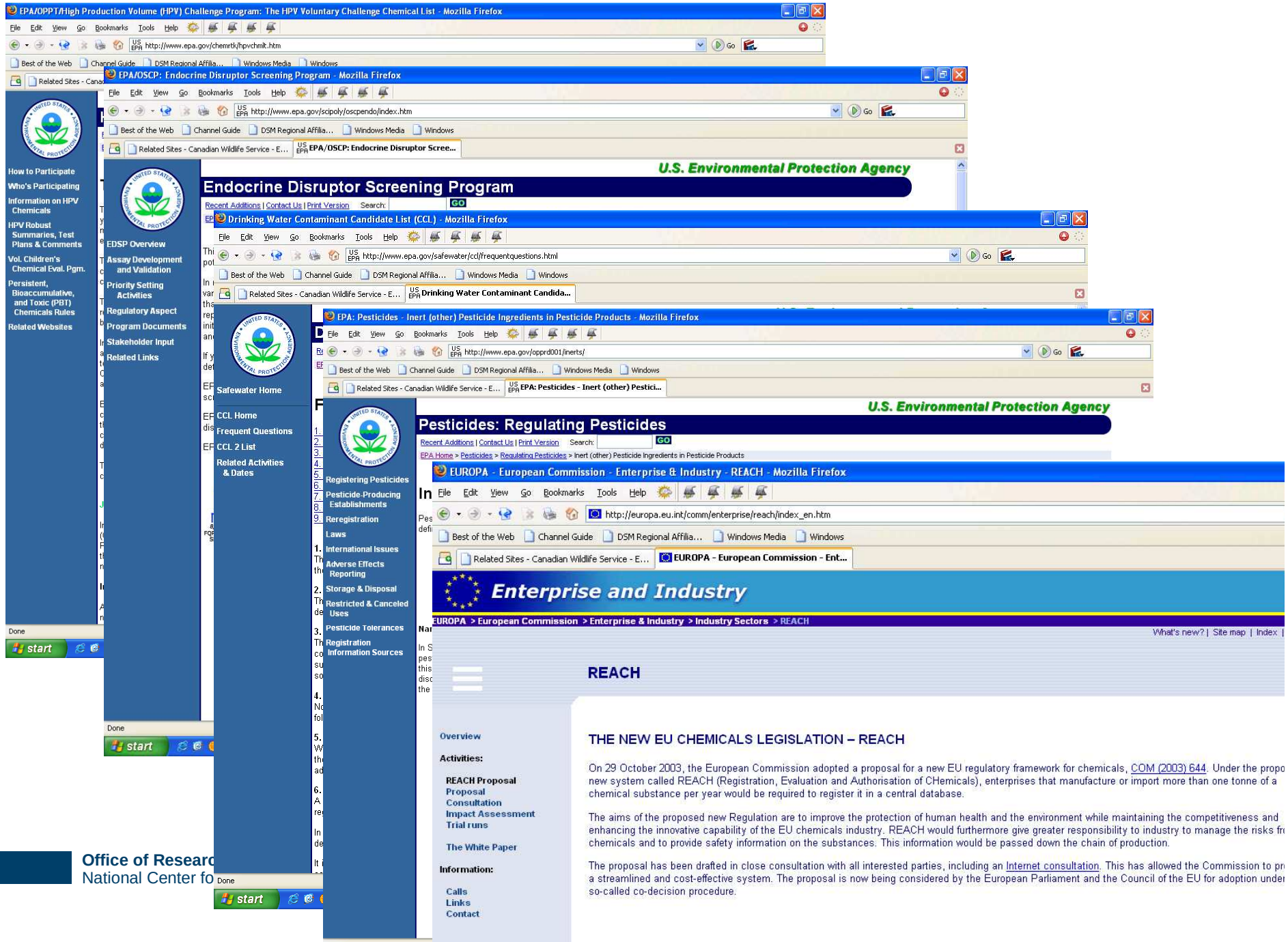
Office of Research and Development
National Center for Computational Toxicology

March 17, 2008

Current Approach for Toxicity Testing

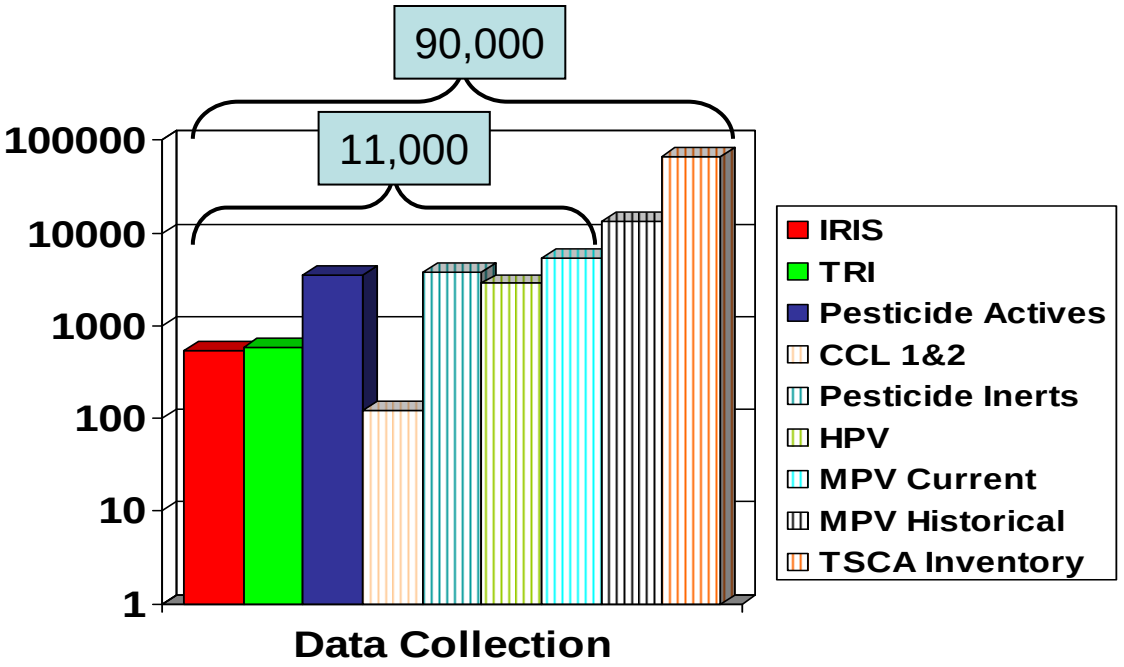
in vivo testing



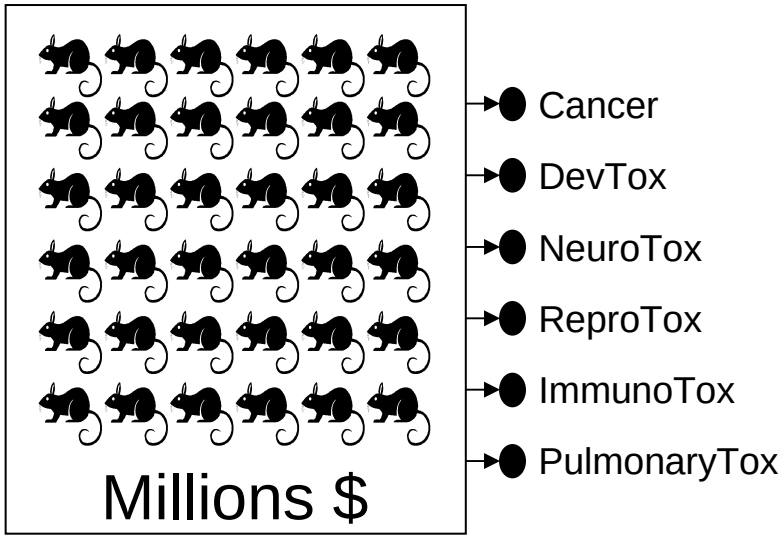

EPA/OPPT/High Production Volume (HPV) Challenge Program: The HPV Voluntary Challenge Chemical List - Mozilla Firefox
http://www.epa.gov/chemrtk/hpvchmlt.htm
How to Participate
Who's Participating
Information on HPV Chemicals
HPV Robust Summaries, Test Plans & Comments
Vol. Children's Chemical Eval. Pgm.
Persistent, Bioaccumulative, and Toxic (PBT) Chemicals Rules
Related Websites
EPA/OSCP: Endocrine Disruptor Screening Program - Mozilla Firefox
http://www.epa.gov/scipoly/oscpendo/index.htm
U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program
EDSP Overview
Assay Development and Validation
Priority Setting Activities
Regulatory Aspect
Program Documents
Stakeholder Input
Related Links
Drinking Water Contaminant Candidate List (CCL) - Mozilla Firefox
http://www.epa.gov/safewater/ccl/frequentquestions.html
Safewater Home
CCL Home
Frequent Questions
CCL 2 List
Related Activities & Dates
EPA: Pesticides - Inert (other) Pesticide Ingredients in Pesticide Products - Mozilla Firefox
http://www.epa.gov/opprd001/inerts/
Pesticides: Regulating Pesticides
Registering Pesticides
Pesticide-Producing Establishments
Reregistration
Laws
International Issues
Adverse Effects Reporting
Storage & Disposal
Restricted & Canceled Uses
Pesticide Tolerances
Registration Information Sources
EUROPA - European Commission - Enterprise & Industry - REACH - Mozilla Firefox
http://europa.eu.int/comm/enterprise/reach/index_en.htm
Enterprise and Industry
EUROPA > European Commission > Enterprise & Industry > Industry Sectors > REACH
REACH
Overview
Activities:
REACH Proposal
Proposal
Consultation
Impact Assessment
Trial runs
The White Paper
Information:
Calls
Links
Contact
THE NEW EU CHEMICALS LEGISLATION – REACH
On 29 October 2003, the European Commission adopted a proposal for a new EU regulatory framework for chemicals, COM (2003) 644. Under the propo
new system called REACH (Registration, Evaluation and Authorisation of CHemicals), enterprises that manufacture or import more than one tonne of a
chemical substance per year would be required to register it in a central database.
The aims of the proposed new Regulation are to improve the protection of human health and the environment while maintaining the competitiveness and
enhancing the innovative capability of the EU chemicals industry. REACH would furthermore give greater responsibility to industry to manage the risks fr
chemicals and to provide safety information on the substances. This information would be passed down the chain of production.
The proposal has been drafted in close consultation with all interested parties, including an Internet consultation. This has allowed the Commission to pr
a streamlined and cost-effective system. The proposal is now being considered by the European Parliament and the Council of the EU for adoption under
so-called co-decision procedure.

Putting Numbers on the Problem

Too Many Chemicals



Too High a Cost



…and not enough data.

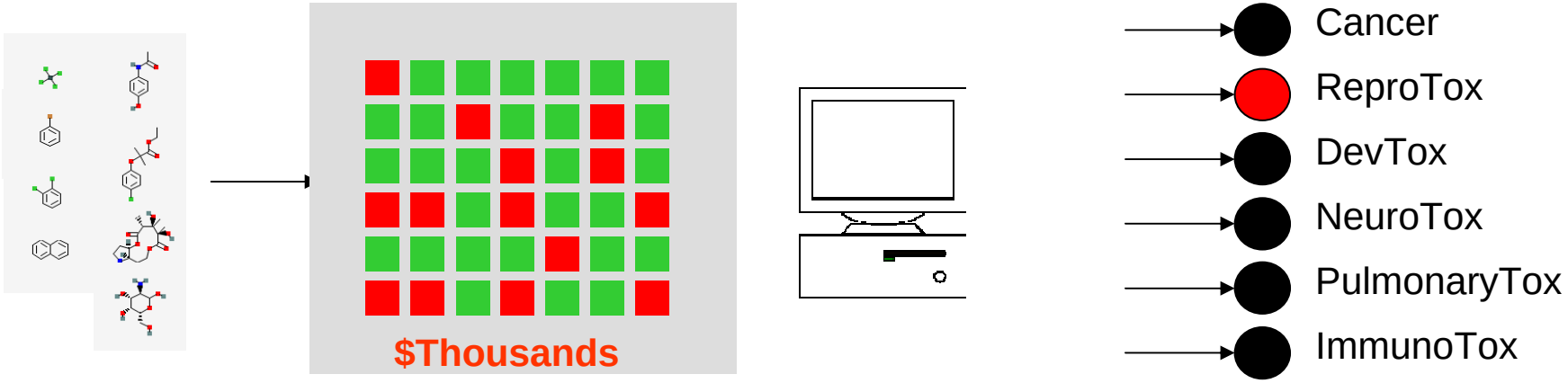
United States
Environmental Protection
Agency

"…to integrate modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals"

www.epa.gov/ncct

Future of Toxicity Testing

in vitro testing *in silico* analysis



HTS
-omics

Bioinformatics/
Machine Learning

EPAs Approach: The ToxCast Research Program

Transforming Toxicology

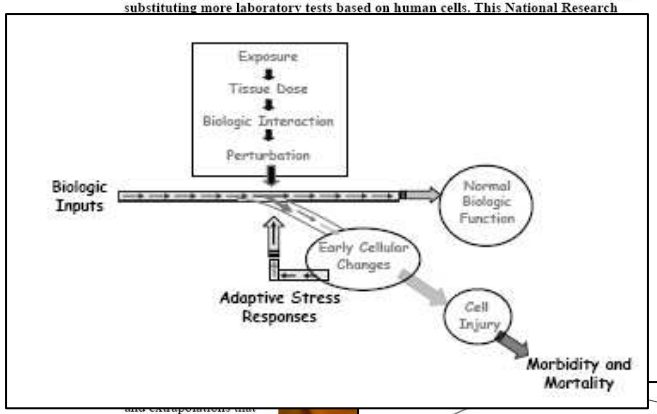
THE NATIONAL ACADEMIES

July 2007

REPORT IN BRIEF

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research



remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about

THE NATIO

National Academy of Sciences • National Academy of

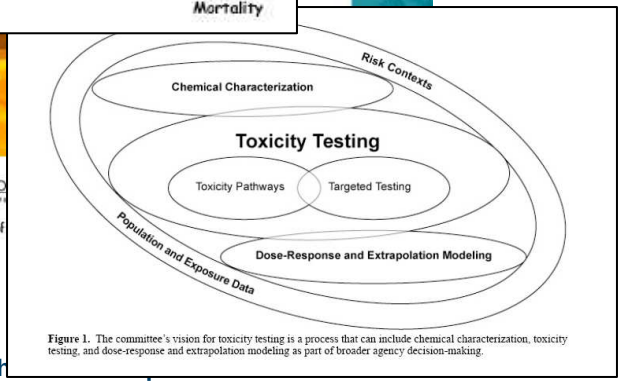


Figure 1. The committee's vision for toxicity testing is a process that can include chemical characterization, toxicity testing, and dose-response and extrapolation modeling as part of broader agency decision-making.

POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

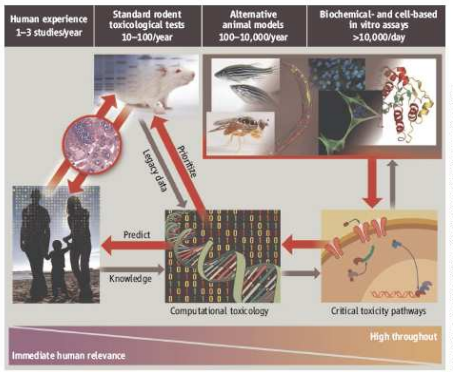
Francis S. Collins,*† George M. Gray,‡* John R. Bucher§*

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,



Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

[1]Director, National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda, MD 20892; [2]Assistant Administrator for the Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460; [3]Associate Director, U.S. National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA.

*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

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Science, Feb 15, 2008

TEHRAN TIMES
IRAN'S LEADING INTERNATIONAL DAILY
Monday, February 18, 2008 | Volume: 10259

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View Rate : 103 # News Code : TTime- 163482 Print Date : Monday, February 18, 2008

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Robots could reduce animal tests

U.S. scientists are taking the first step towards testing potentially hazardous chemicals on cells grown in a laboratory, without using live animals.

Two government agencies are looking into the merits of using high-speed automated robots to carry out tests.

The long-term goal is to reduce the cost, time and number of animals used in screening everything from pesticides to household chemicals.

The move follows calls for scientists to rely less on animal studies.

Robots would be able to carry out hundreds of thousands of chemical tests a day to identify chemicals with toxic effects.

Details were published in the journal Science and discussed at the annual meeting of the American Association for the Advancement of Science (AAAS) in Boston.

Faster and cheaper

Speaking in a live link-up, Dr. Francis Collins, Director of the National Human Genome Research Institute at the National Institute of Health (NIH), said

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Phased Development of ToxCast

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
I	320	Data Rich (pesticides)	Signature Development	>400	$20k	FY07-08
IIa	>300	Data Rich Chemicals	Validation	>400	$15-20k	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	$15-20k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	$15-20k	FY10
III	Thousands	Data poor	Prediction and Prioritization	???	$10-15k	FY11-12

- Affordable science-based system for categorizing chemicals
- Increasing confidence as database grows
- Identifies potential mechanisms of action
- Refines and reduces animal use for hazard ID and risk assessment

ToxCast Website: www.epa.gov/ncct/toxcast

ToxCast™ Program

Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

Introduction

In 2007, EPA launched ToxCast™ in order to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time. Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast™ is building computational models to forecast the potential human toxicity of chemicals. These hazard predictions will provide EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, and lead to more efficient use of animal testing.

ToxCast™ Navigation

- Introduction
- ToxCast™ Chemicals
- ToxCast™ Assays
- ToxCast™ Information Management
- ToxCast™ Partnerships
- ToxCast™ Contractors
- ToxCast™ Presentations
- ToxCast™ Publications
- ToxCast™ News

In its first phase, ToxCast™ is profiling over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints. These endpoints include biochemical assays of protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebrafish embryos. Almost all of the compounds being examined in Phase 1 of ToxCast™ have been tested in traditional toxicology tests, including developmental toxicity, multi-generation studies, and sub-chronic and chronic rodent bioassays. ToxRefDB, a relational database being created to house this information, will contain nearly $1B worth of toxicity studies in animals when completed. ToxRefDB is integrated into a more comprehensive data management system developed by NCCT called ACToR (Aggregated Computational Toxicology Resource), that manages the large-scale datasets of ToxCast™. ACToR is comprised of several independent data repositories linked to a common database of chemical structures and properties, and to tools for development of predictive HTS and genomic bioactivity signatures that strongly correlate with specific toxicity endpoints from ToxRefDB. These ToxCast™ signatures will be defined and evaluated by their ability to predict outcomes from existing mammalian toxicity testing, and identify toxicity pathways that are relevant to human health effects.

The second phase of ToxCast™ will screen additional compounds representing broader chemical structure and use classes, in order to evaluate the predictive bioactivity signatures developed in Phase I. Following successful conclusion of Phases I and II, ToxCast™ will provide EPA regulatory programs an efficient tool for rapidly and efficiently screening compounds and prioritizing further toxicity testing.

United States Environmental Protection Agency

Key Components of a Proof of Concept

- Assays covering Toxicity Pathways
- Chemicals
- Linkage to Traditional Phenotype Findings
- Data Analysis and Interpretation

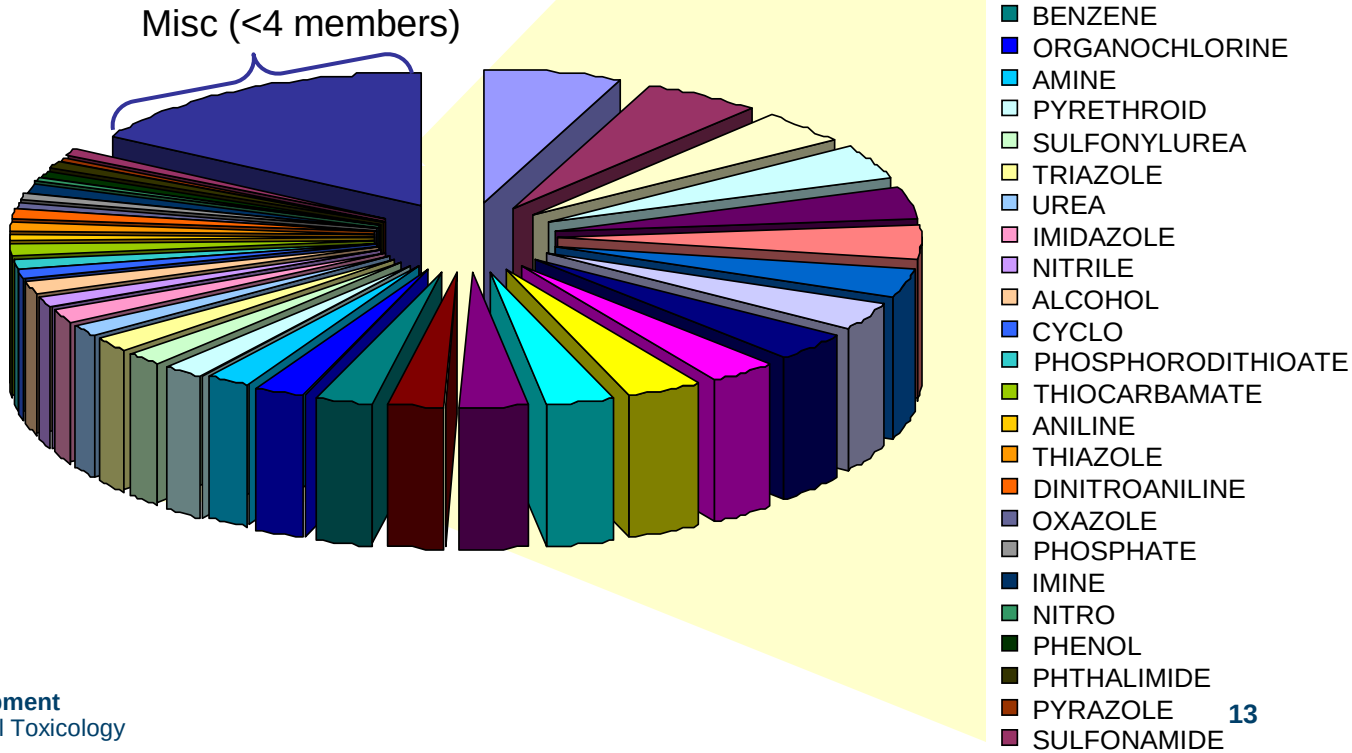
The ToxCast Team

United States Environmental Protection Agency

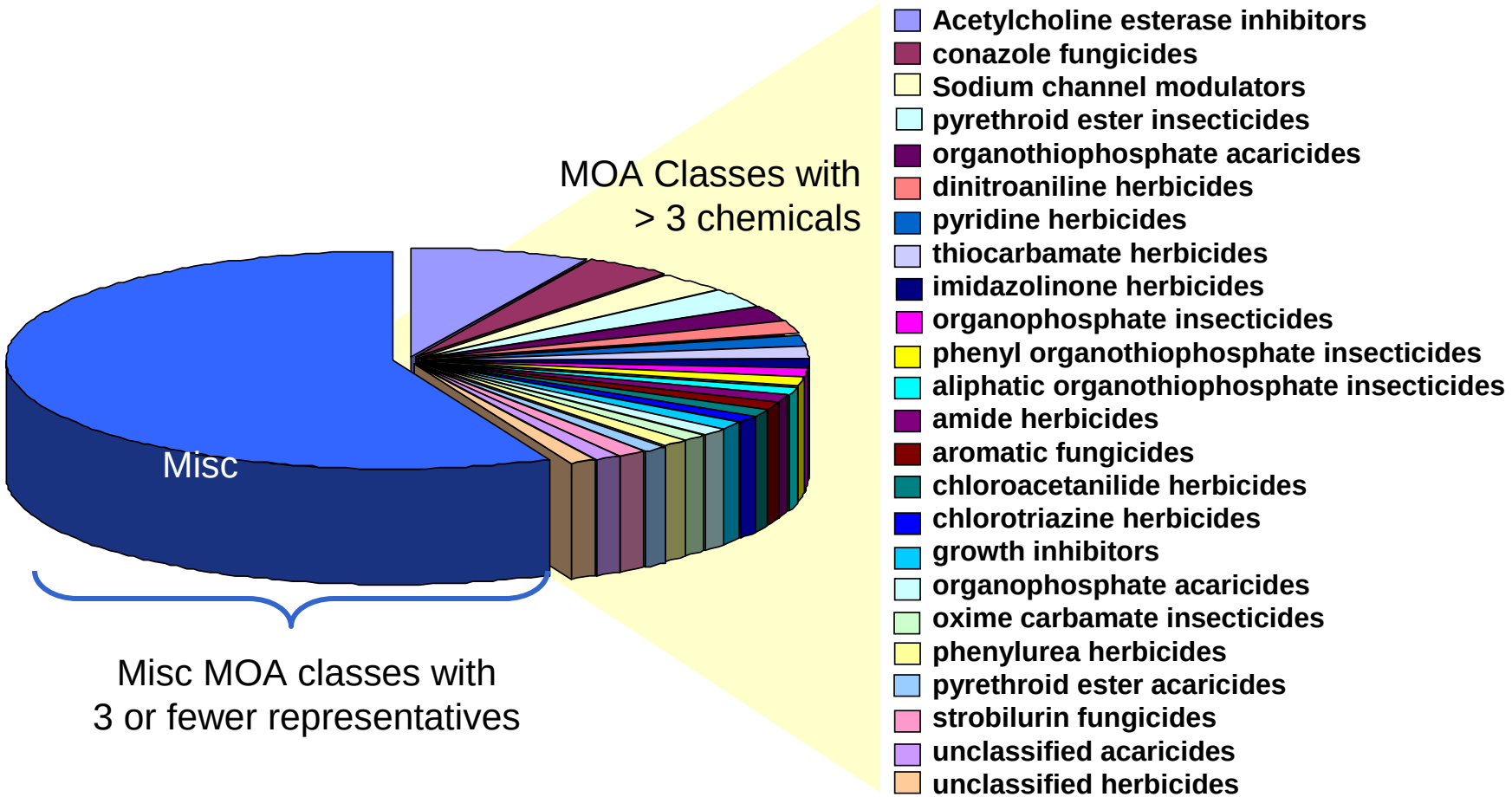
The Chemicals

Chemical Classes in ToxCast_320

- 309 Unique Structures
- Replicates for QC
- 291 Pesticide Actives
- 9 Industrial Chemicals
- 8 Metabolites
- 56/73 Proposed Tier 1 EDSP
- 14 HPV
- 11 HPV Challenge



Mode-of-Action Classes in ToxCast_320



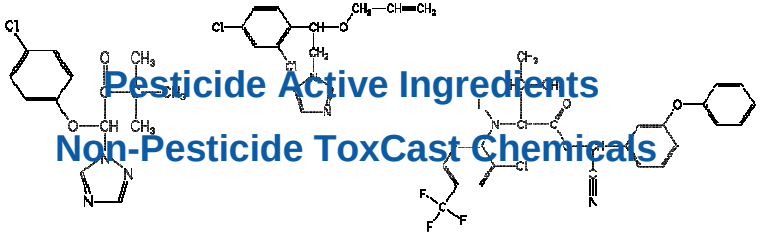
Classification based on OPPIN

United States Environmental Protection Agency

The Toxicity Data

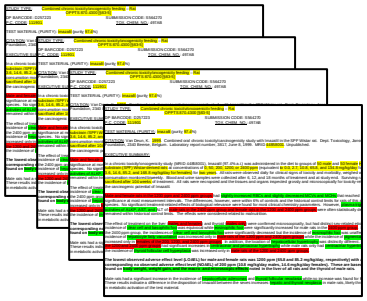
ToxRefDB: Capturing the Legacy Data

- **Chemical & Toxicity Coverage**



- **Source Data**

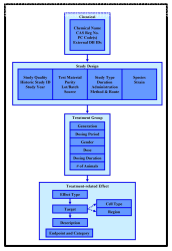
DER



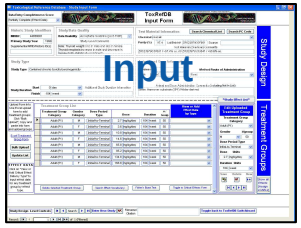
- **Database**

ToxRefDB

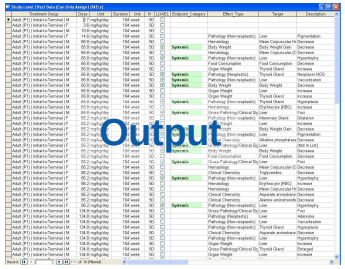
Structure



Input



Output



- **Applications**

RETRO SPEC TIVE

Research

Extraction of DER information

United States Environmental Protection Agency

Chemical Info
Study Design
Treatment Group Info
Treatment-related Effects
Endpoint/ Critical Effects

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding – Rat
OPPTS 870.4300 [§83-5]

DP BARCODE: D257223
P.C. CODE: 111901

SUBMISSION CODE: S564270
TOX. CHEM. NO.: 497AB

TEST MATERIAL (PURITY): Imazalil (purity ≥97.4%)
SYNONYMS: R023979

CITATION: Van Deun, K. 1999. Combined oral chronic toxicity/carcinogenicity study with Imazalil in the SPF Wistar rat. Dept. Toxicology, Janssen Res 2340 Beerse, Belgium. Laboratory report number, 3817, June 8, 1999. MRID 44858001. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., 2340 Beerse, Belgium

EXECUTIVE SUMMARY:

In a chronic toxicity/oncogenicity study (MRID 44858001), Imazalil (≥97.4% a.i.) was administered in the diet to groups of 50 male and 50 female Hannover substrain (SPF) Wistar-derived rats at concentrations of 0, 50, 200, 1200, or 2400 ppm (equivalent to 0.0, 2.7, 10.8, 65.8, and 134.8 mg/kg/day for males and 0.0, 3.6, 14.6, 85.2, and 168.8 mg/kg/day for females) for two years. All rats were observed daily for clinical signs of toxicity and morbidity, weighed weekly, and food consumption monitored biweekly. Blood and urine samples were collected after 6, 12, and 18 months of treatment and at study end. Surviving rats were sacrificed after 104 weeks of treatment. All rats were necropsied and the tissues and organs inspected grossly and microscopically for toxicity-related effects and the carcinogenic potential of Imazalil.

The absolute weights of most organs were decreased while their weights relative to body weight increased for male and female rats in the 1200 and 2400 ppm treatment groups. These effects are considered related to inanition and inappetence and not a direct result of Imazalil treatment. However, effects found in the liver and thyroid was considered directly related to treatment. The absolute liver weight of male rats in the 2400 ppm group was increased while it was decreased in female rats. The associated relative liver weights of male and female rats in the 1200 and 2400 ppm groups were significantly increased 9-26%. In addition, the absolute and relative thyroid weights of male but not female rats in the 1200 and 2400 ppm groups were increased.

The effect of treatment on the liver (males and females) and thyroid (males only) were confirmed microscopically, but had distinct sex-related etiologies. The incidence of clear cell and basophilic foci was equivocal while eosinophilic foci were significantly increased for male rats in the 2400 ppm group. In female rats of the 2400 ppm group, the incidences of clear cell and basophilic foci were significantly decreased but the incidence of eosinophilic foci was unaffected. Also, the incidence of hepatocyte fatty vacuolation was increased only in male rats of the 1200 ppm and 2400 ppm groups while the incidence of pigmentation was increased only in females of the 200, 1200, and 2400 ppm groups. In addition, the location of hepatocellular hypertrophy was distinctly different. Female rats in the 1200 and 2400 ppm groups had significant increases in centriacinar and periacinar hypertrophy while male rats only had centriacinar hypertrophy. Finally, the incidence of thyroid follicular cell hyperplasia was increased only in male rats of the 1200 and 2400 ppm groups.

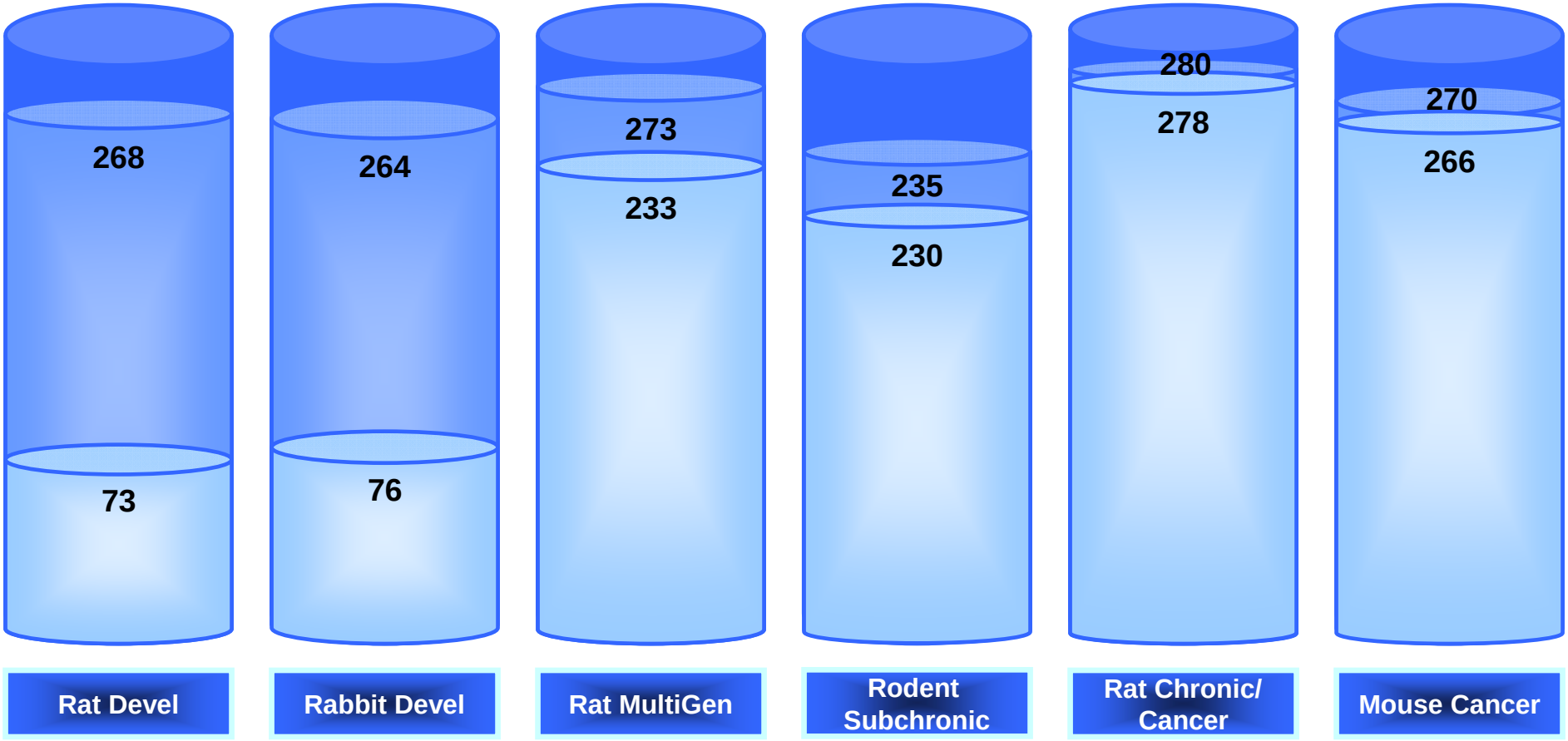
The lowest observed adverse effect level (LOAEL) for male and female rats was 1200 ppm (65.8 and 85.2 mg/kg/day, respectively) with a corresponding no observed adverse effect level (NOAEL) of 200 ppm (10.8 mg/kg/day males, 14.6 mg/kg/day females). These are based on the effects found on body weight, weight gain, and the macro- and microscopic effects noted in the liver of all rats and the thyroid of male rats.

Male rats had a significant increase in the incidence of hepatocellular adenomas and thyroid follicular neoplasia while no increase was found for female rats. These results indicate a difference in the disposition of Imazalil between the sexes increases hepatic and thyroid neoplasia in male rats, likely through differences in metabolic activation of the test material.

This chronic toxicity/oncogenicity study in the rat is **Acceptable/guideline** and satisfies the guideline requirement for a combined chronic toxicity/oncogenicity study in rats [83-5]. No deficiencies were noted for this study.

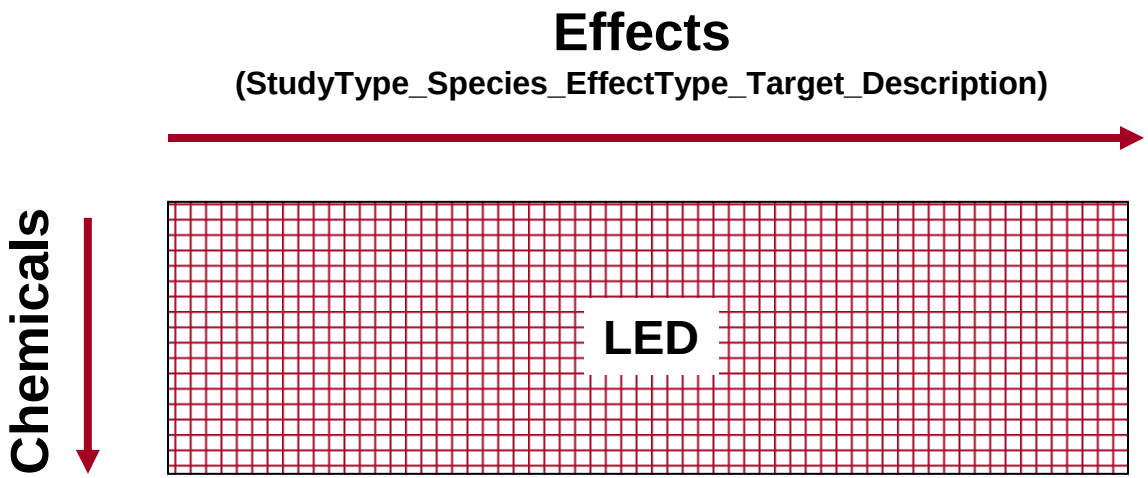
Data Entry Status

ToxCast Phase I Chemicals Only
Total: 291 Pesticides



Chemical Classification

- Created directly from ToxRefDB output
- Combination of:
  - Chemical (CAS No. & Chemical Name)
  - Effect
    - **Study Type** (Chronic, Multigeneration, Developmental)
    - **Species** (Rat, Mouse, Rabbit)
    - **Effect Type** (Body weight, Clinical Chemistry, Hematology, Pathology, etc.)
    - **Effect Target** (Target-organ, ALT, AST, ALP, Cholesterol, etc.)
    - **Effect Description** (Increase, Decrease, Hypertrophy, Hyperplasia, Adenoma)
    - *Aggregated Effects – Collection of related individual effects*
  - LED (Lowest Effective Dose)
    - Not used as regulatory term
    - Minimum dose of observed effect



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Summary Statistics

Subchronic Rat, Chronic/Cancer Rat, and Cancer Mouse Studies

	Chemicals	Studies	Treatment Groups	Treatment Groups with Effects	Effects[a]	Critical Effects[b]
Total	***334***	***831***	***9,466***	***4,431***	***31,427***	***4,865***
Subchronic Rat	236	251	2,179	1,370	11,796	1,739
Chronic/Cancer Rat	281	300	4,228	1,721	12,215	1,822
Cancer Mouse	266	280	3,059	1,340	7,416	1,304

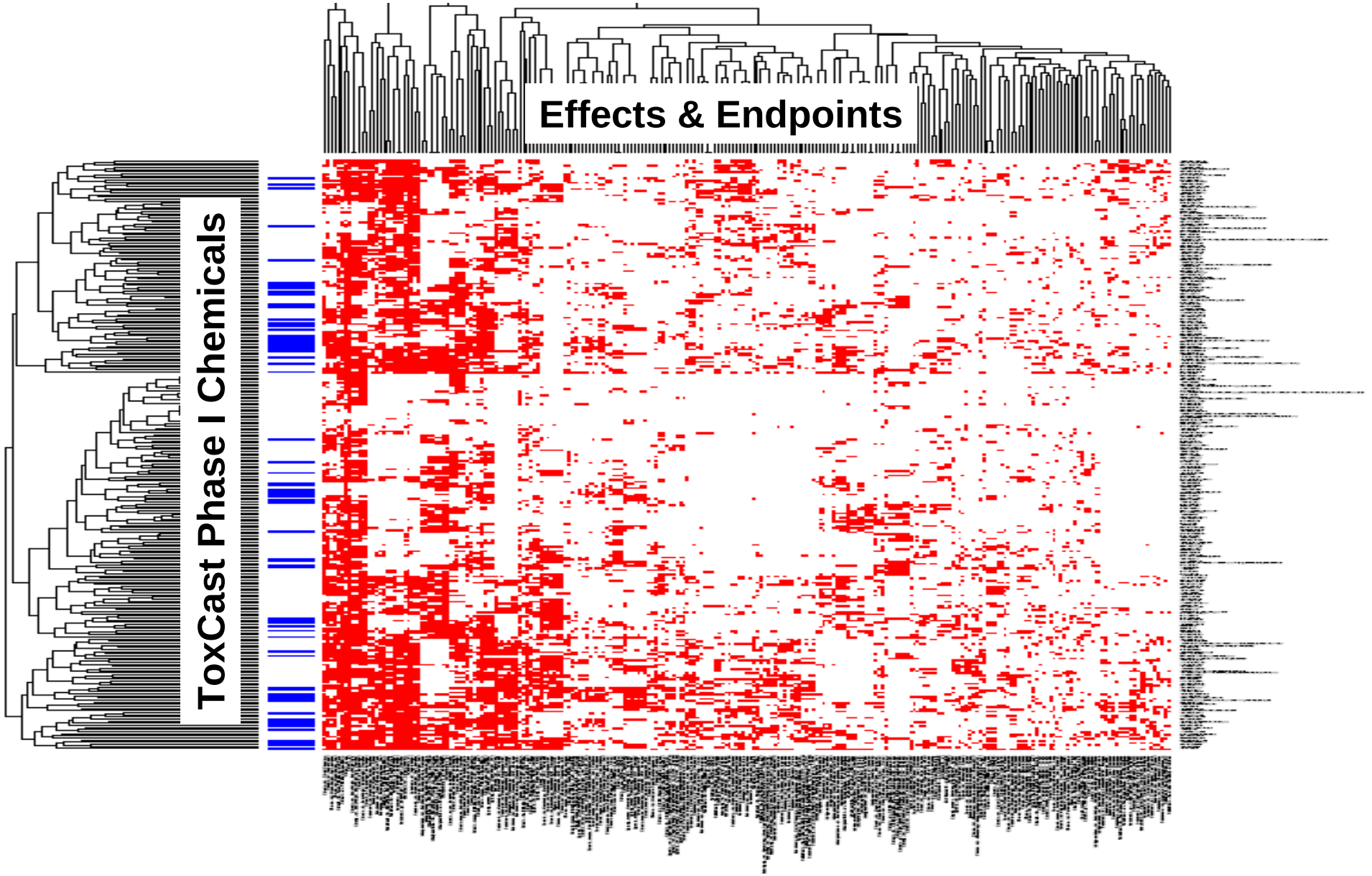
(a) - Total number of effect type, target, and description combinations assigned to any treatment group

(b) - Effects that are criteria for establishing the systemic LOAEL

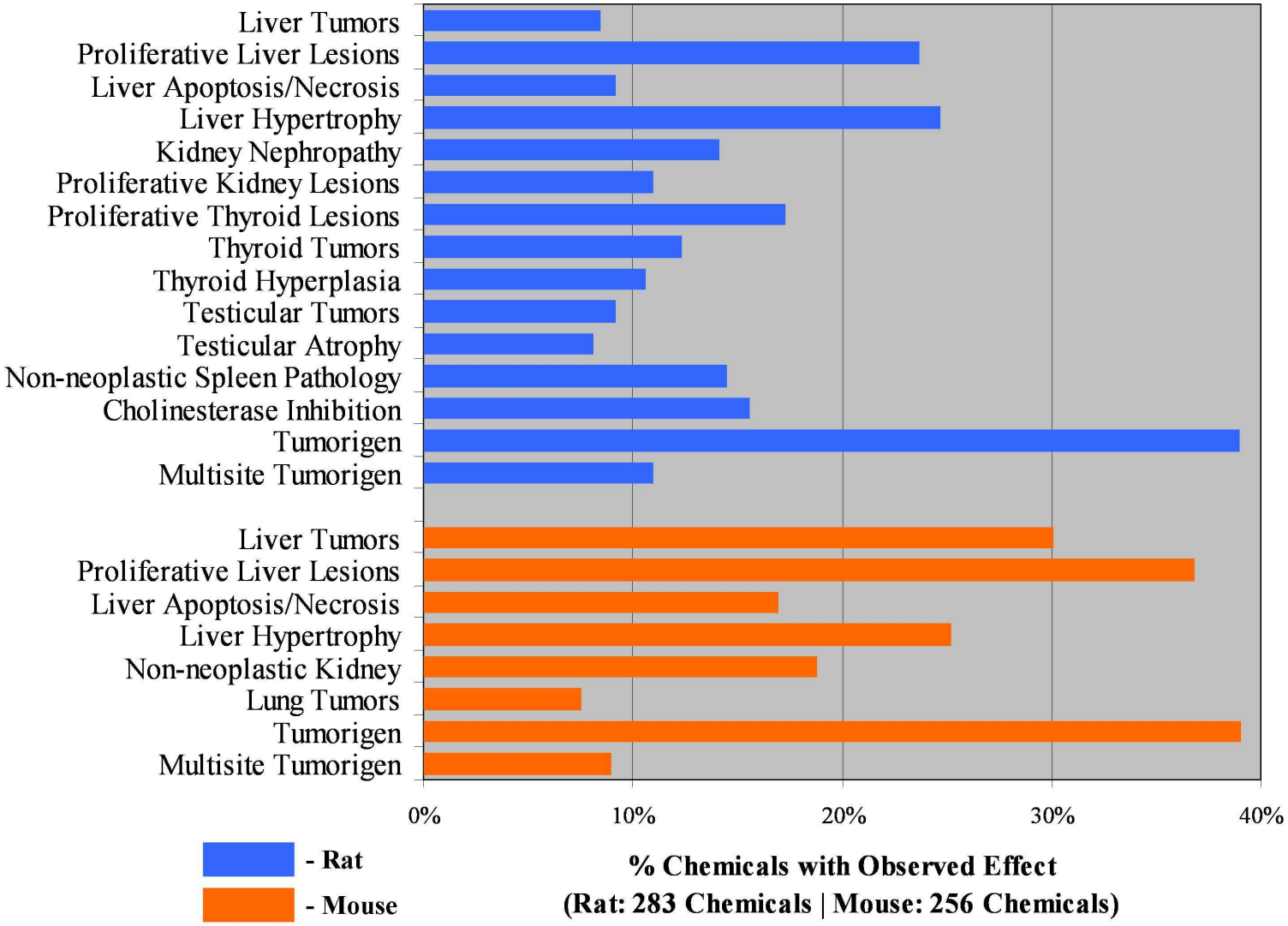
>9,000 Treatment groups
46% with at least one effect
31,000 Total effects
~5,000 Total Effects at the LED
~1,300 Different types of effects

$400 Million Dollars Worth of *In Vivo* Chronic/Cancer Bioassay Effects and Endpoints

EPA United States Environmental Protection Agency



Common Phenotypes in Chronic Rodent Studies



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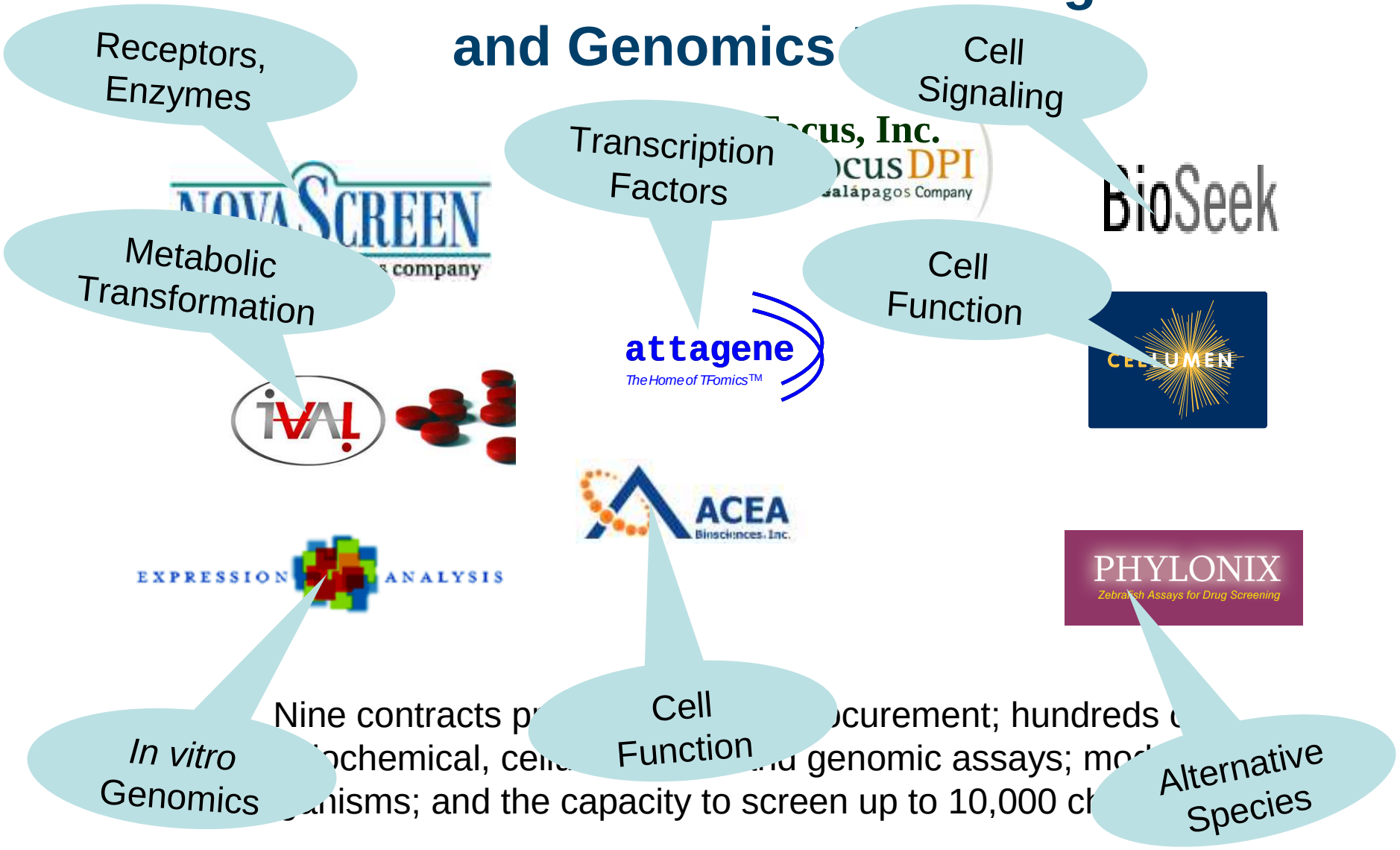
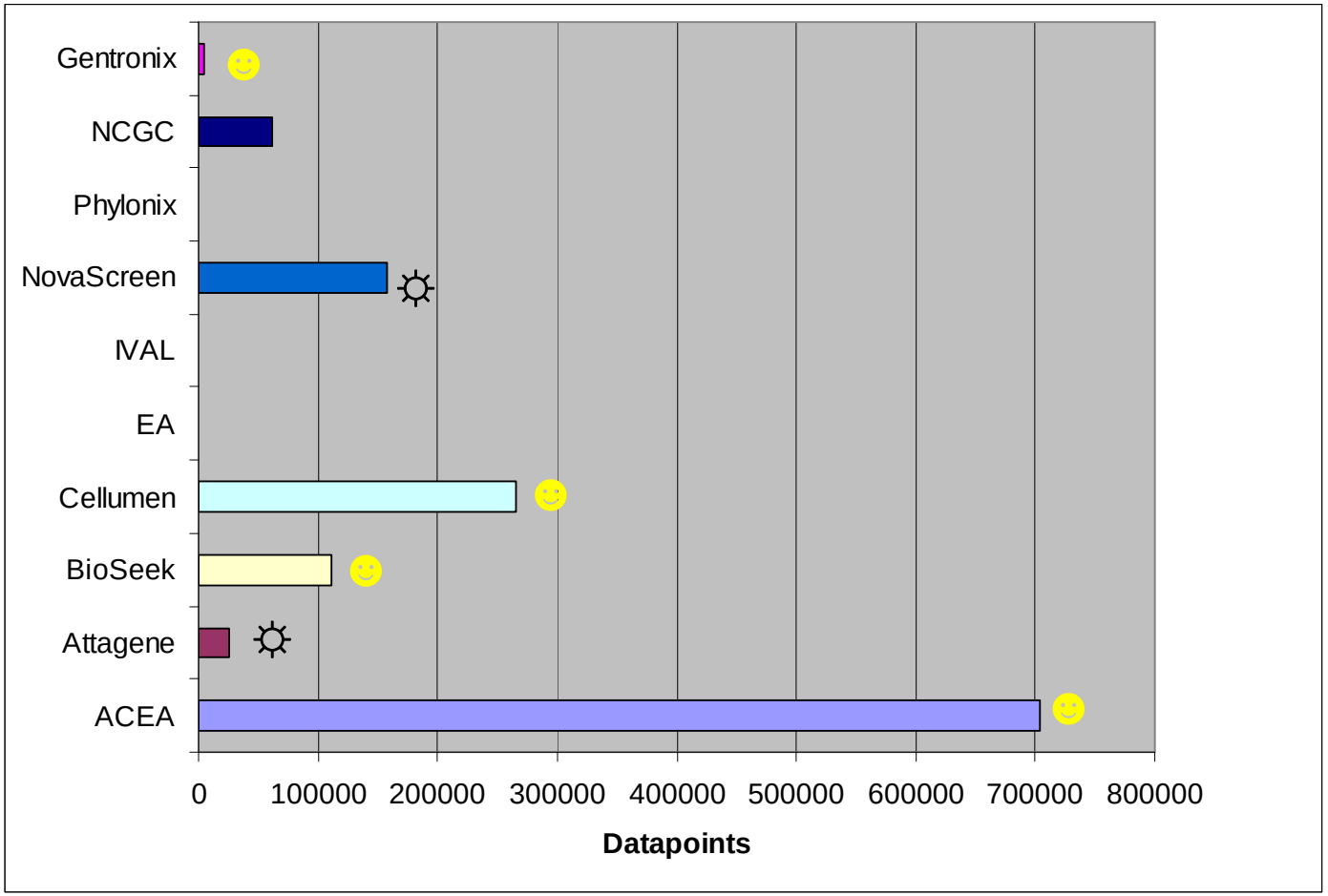
The HTS Assays and Toxicity Pathways

ToxCast Contracts for Generating HTS and Genomics



Nine contracts p… …ocurement; hundreds …
…ochemical, cell… …d genomic assays; mo…
…anisms; and the capacity to screen up to 10,000 ch…

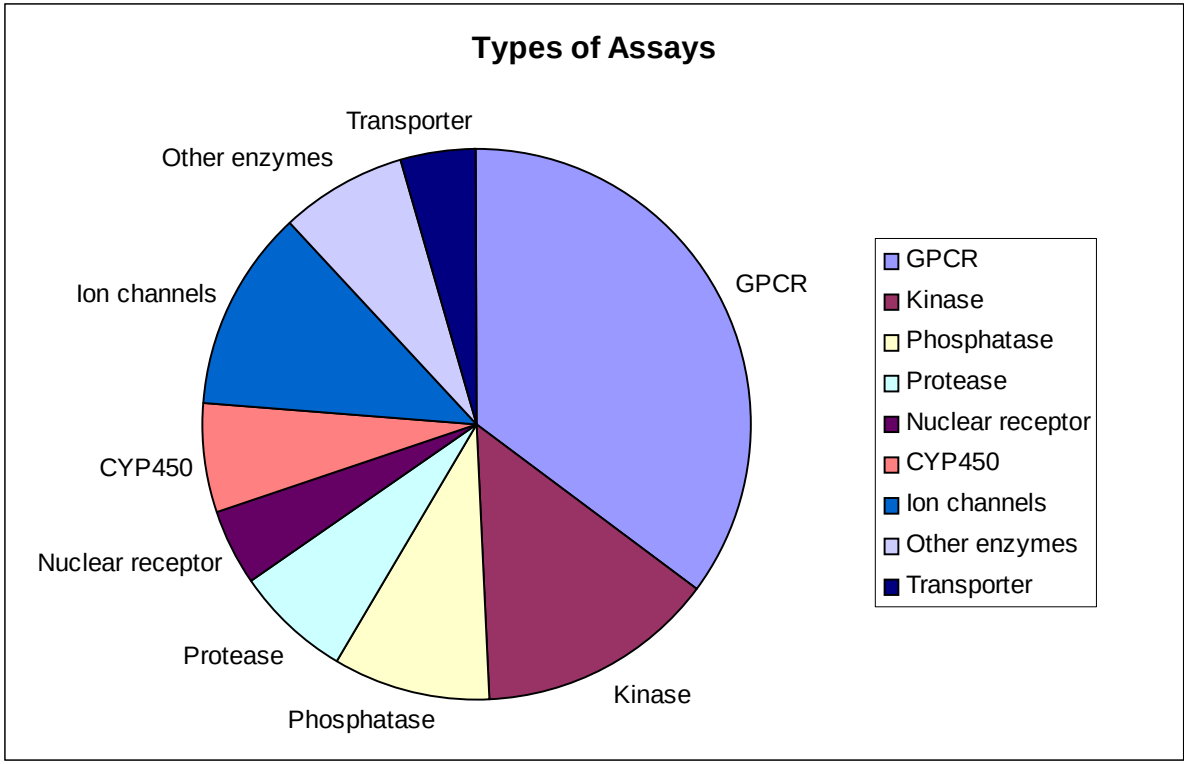
The Deluge of Data has Started……..

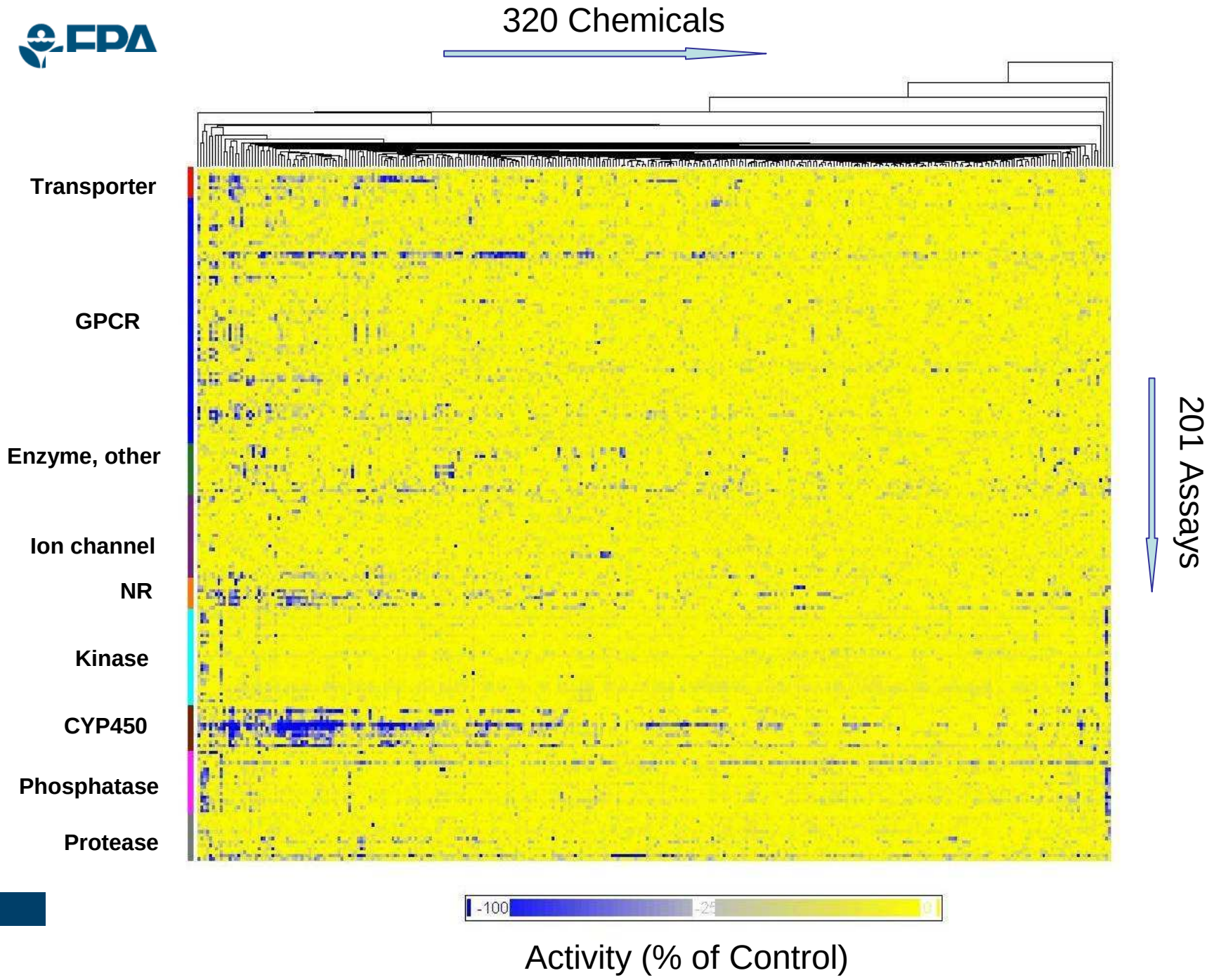


☺ Data acquisition completed; ☼ Concentration response follow up underway

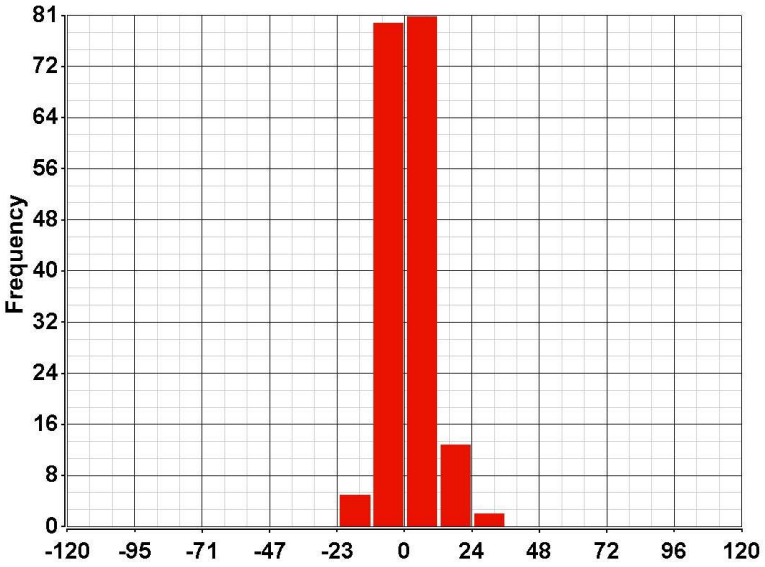
ToxCast Biochemical Assays

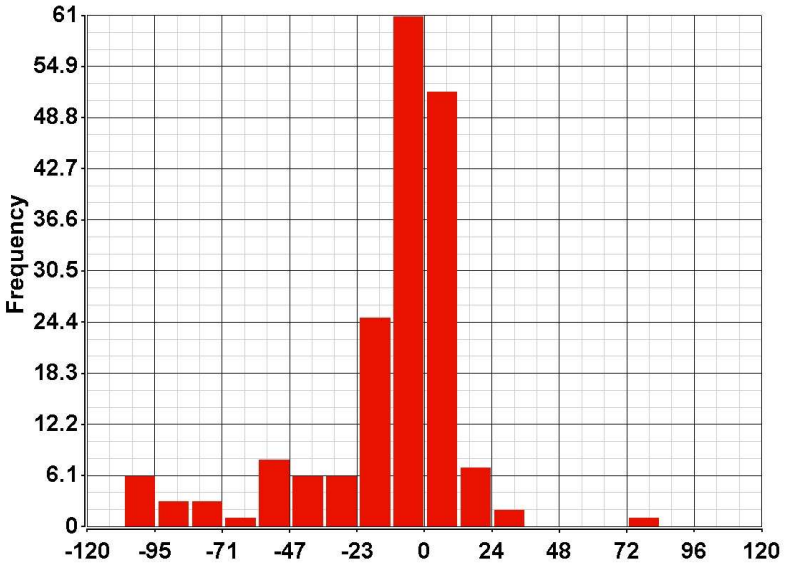
- NovaScreen/Caliper LifeSciences
- 200+ Assays
- Variety of assay technologies:
  - Receptor binding
  - Enzyme inhibition
  - Microfluidic
  - Fluorescent
  - Radioligand
  - Colorimetric

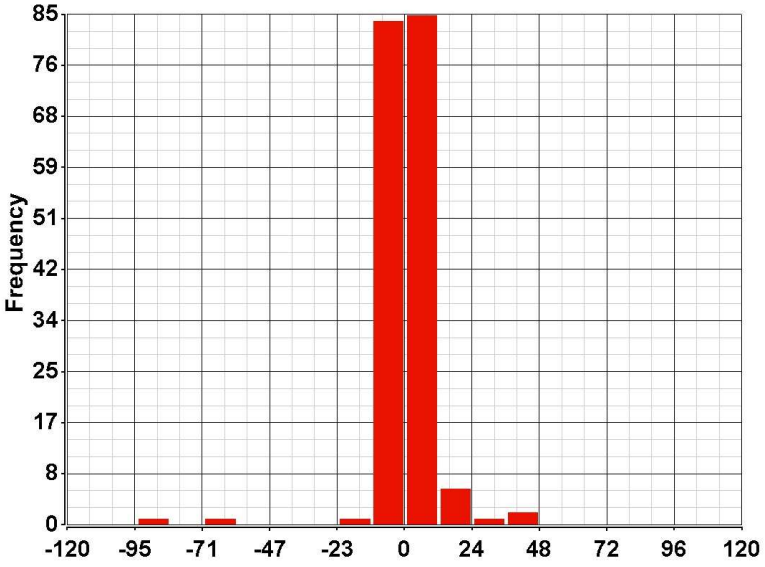


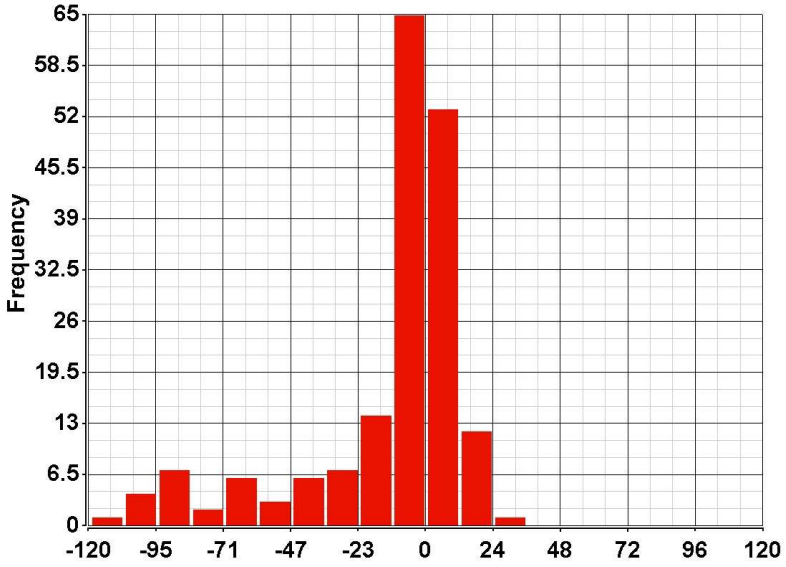

320 Chemicals
Transporter
GPCR
Enzyme, other
Ion channel
NR
Kinase
CYP450
Phosphatase
Protease
201 Assays
-100
Activity (% of Control)

Examples of Chemical Activity Patterns

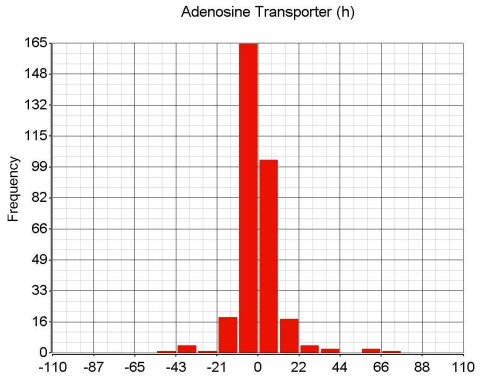


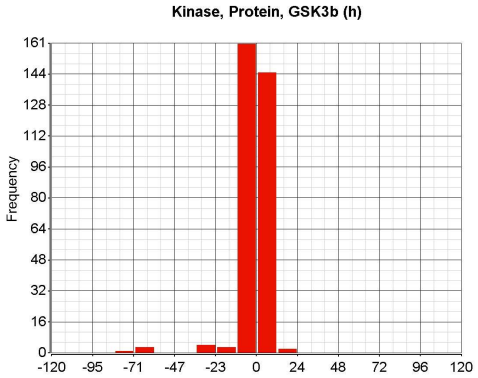


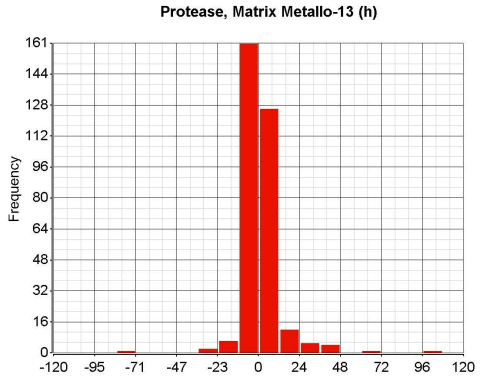


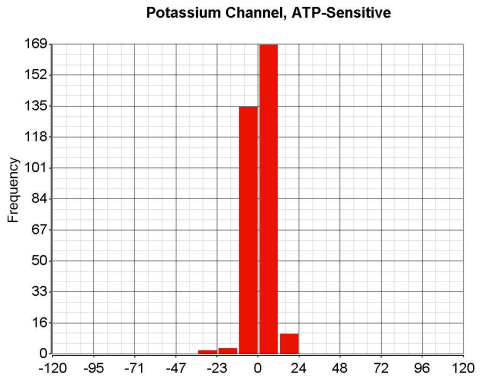


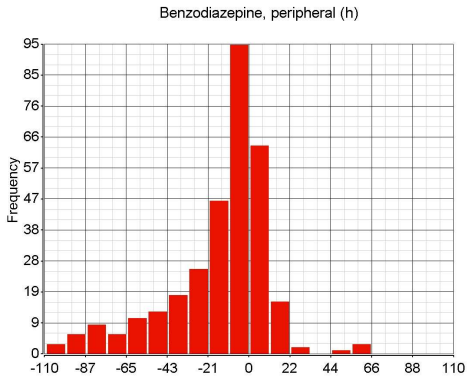
Example Biochemical Assay Results

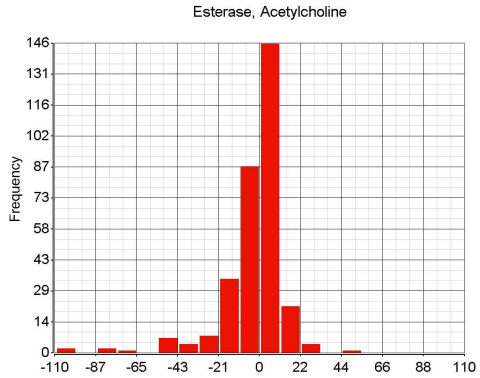


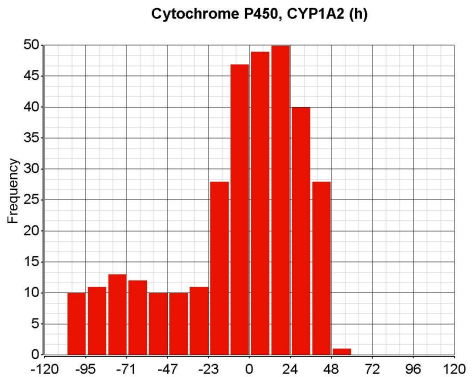


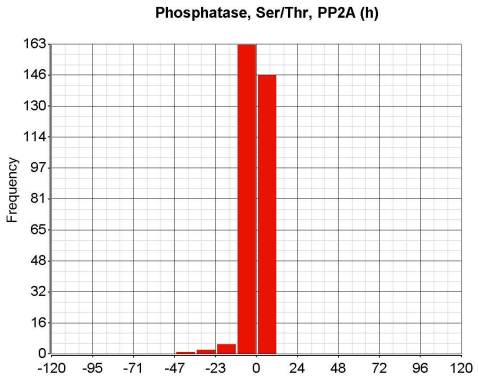


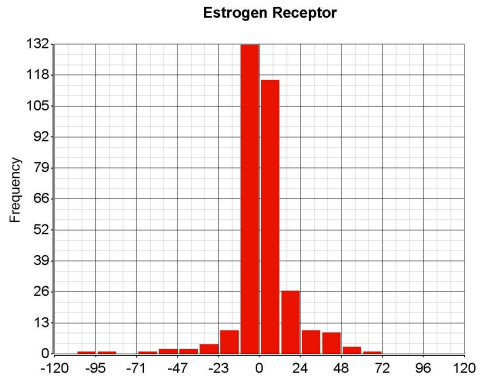




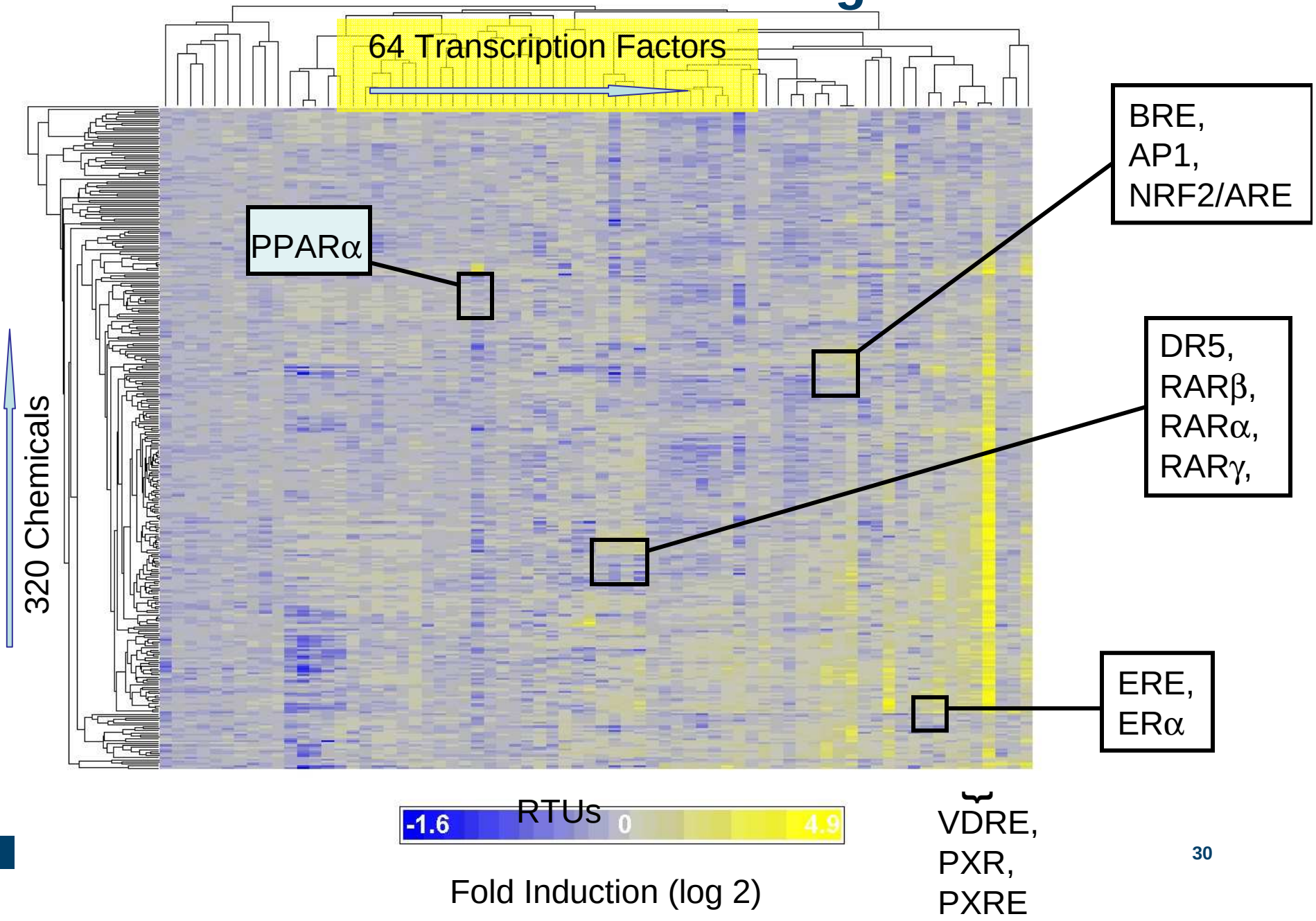








Hierachical Cluster Attagene Results



Evolution of Phase I

- **ToxCast 1.0 (April, 2007)**
  - *Enzyme inhibition/receptor binding HTS (Novascreen)*
  - *NR/transcription factors (Attagene, NCGC)*
  - *Cellular impedance (ACEA)*
  - *Complex cell interactions (BioSeek)*
  - *Hepatocelluar HCS (Cellumen)*
  - *Hepatic, renal and airway cytotoxicity (IVAL)*
  - *In vitro hepatogenomics (IVAL, Expression Analysis)*
  - *Zebrafish developmental toxicity (Phylonix)*
- **ToxCast 1.1 (January, 2008)**
  - *Neurite outgrowth HCS (NHEERL)*
  - *Cell proliferation (NHEERL)*
  - *Zebrafish developmental toxicity (NHEERL)*
- **ToxCast 1.2 (March, 2008)**
  - *Organ culture: liver, kidney, lung (Hamner Institutes)*
  - *HTS Genotoxicity (Gentronix)*
  - *Toxicity and signaling pathways (Invitrogen)*
  - *NR Activation and translocation (CellzDirect)*
  - *3D Cellular microarray with metabolism (Solidus)*

8 Assay Sources & 412 Endpoints

+3 Assay Sources & 16 Endpoints

+5 Assay Sources & 32 Endpoints

16 Assay Sources, 460 Endpoints

United States Environmental Protection Agency

Analysis and Interpretation

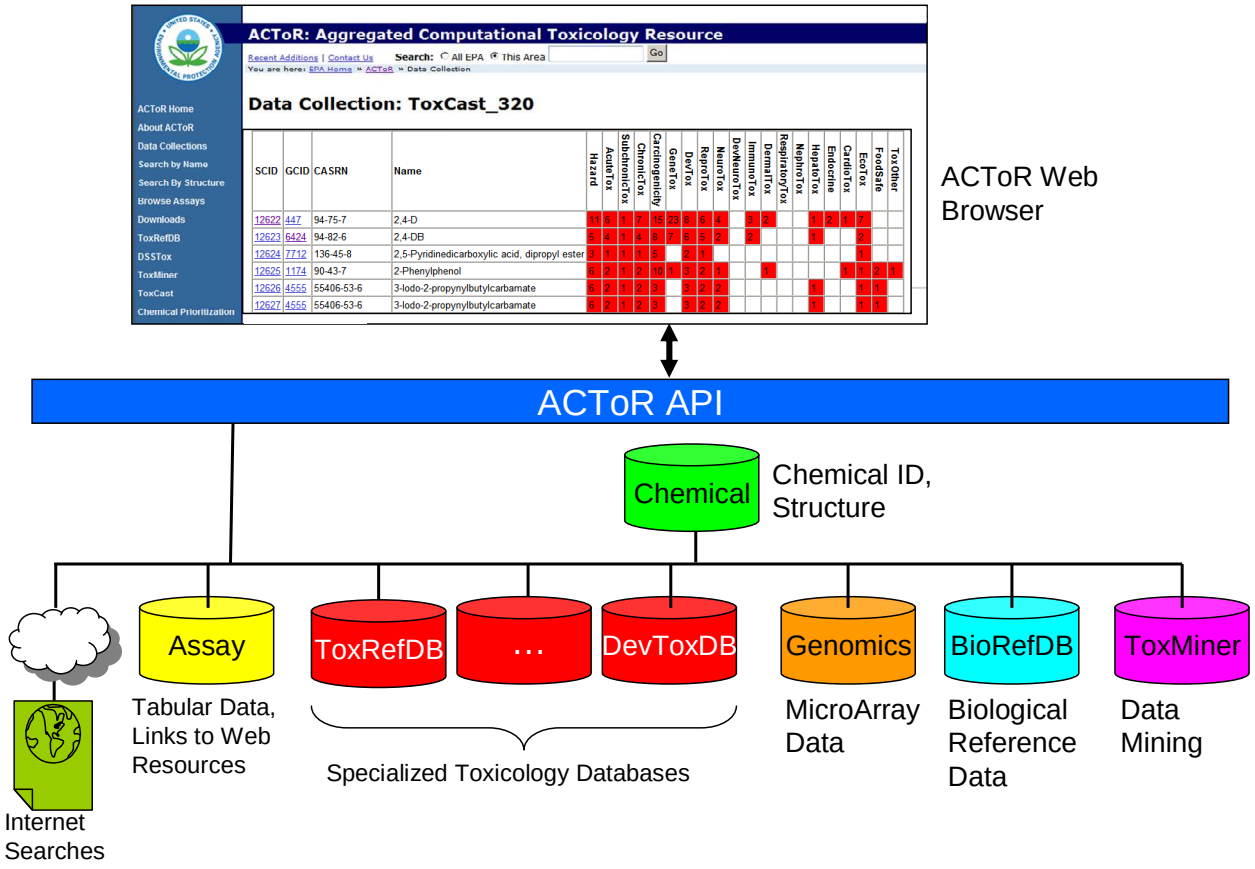
ToxCast Phase I Data Matrix

	Chemical			HTS			HCS			Genomics			ToxRefDB		
Chemical	Physico-Chemical Properties			In-vitro / Biochemical Assays			Cellular Assays			Gene Expression Signatures			Toxicity Endpoints		
	P1	…	PN	A1	…	AN	C1	…	CN	S1	…	SN	T1	…	TN
C1															
C2															
C3															
…															
CN															

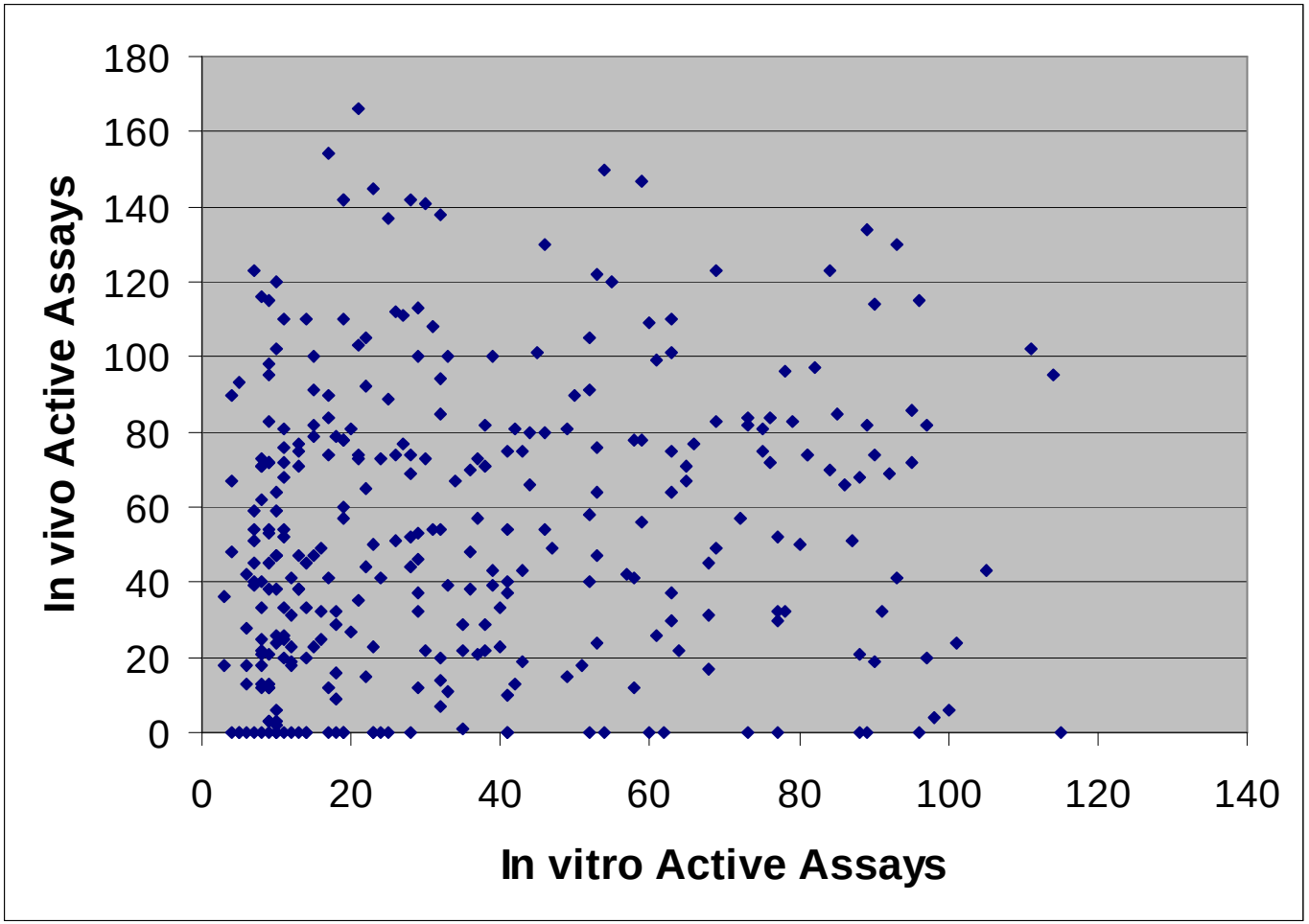
Data is both quantitative and categorical

United States Environmental Protection Agency

ACToR: Aggregated Computational Toxicology Resource



Does Overall In Vitro Activity Signify In Vivo Toxicity?



Attagene, BioSeek, NovaScreen

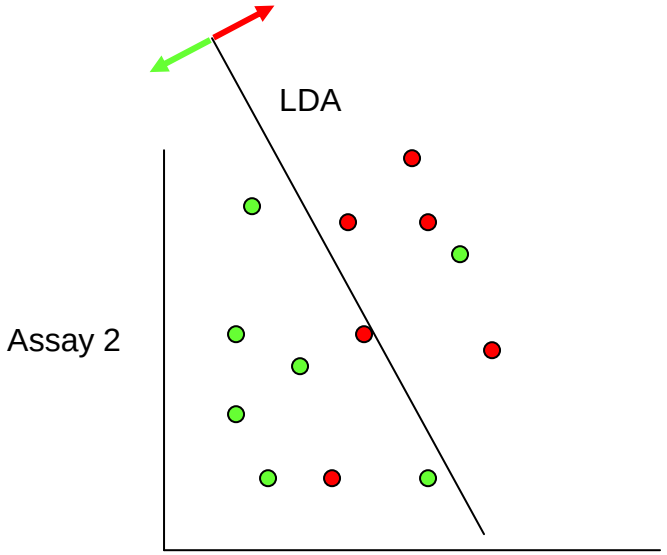
Comparing Activities by Chemical Class

One Chemical Class vs. NovaScreen Assays

NAME	CYP2C19	CYP2C9	CYP3A1	Dopamine Transporter (Human)	CYP2D2	Androgen Receptor	Dopamine Transporter (Rat)	CYP2B6	CYP2D1	CYP3A4	Progesterone Receptor	Benzodiazepine Receptor
Chemical 1	1	1	1	1	1	0	1	0	0	1	0	0
Chemical 2	1	1	1	1	1	0	0	1	1	0	0	0
Chemical 3	1	1	1	0	1	0	0	0	1	1	1	0
Chemical 4	1	1	0	0	0	0	0	0	0	1	0	0
…..	1	1	1	0	1	1	0	1	1	NA	1	1
…..	1	1	1	1	1	0	1	1	1	NA	1	0
…..	1	1	1	1	1	1	1	1	1	1	1	1
…..	1	1	1	1	0	0	0	0	0	NA	0	0
…..	1	0	1	1	0	1	1	0	1	1	0	0
…..	1	1	1	1	1	1	1	1	1	NA	1	1
…..	1	1	1	0	0	0	0	1	0	NA	0	1
…..	1	1	1	0	1	1	0	1	0	1	1	0
…..	1	1	0	1	1	1	1	0	0	1	0	1
…..	1	0	0	1	0	1	1	0	0	0	0	0
…..	1	1	1	1	1	1	0	1	1	1	1	1
Chemical 16	1	1	1	1	0	1	1	0	0	NA	0	0
Totals	**16**	**14**	**13**	**11**	**10**	**9**	**8**	**8**	**8**	**8**	**7**	**6**

Association Analysis / Signatures

- Use Machine Learning methods
  - SLR: Stepwise Logistic Regression
  - LDA: Linear Discriminant Analysis
  - SVM: Support Vector Machines
  - Many others
- For each binary endpoint, build models of form
  - *Predictor* = *F*(assay values)
  - If
    - *Predictor* for a chemical meets criteria
  - Then
    - Predict endpoint to be positive for the chemical



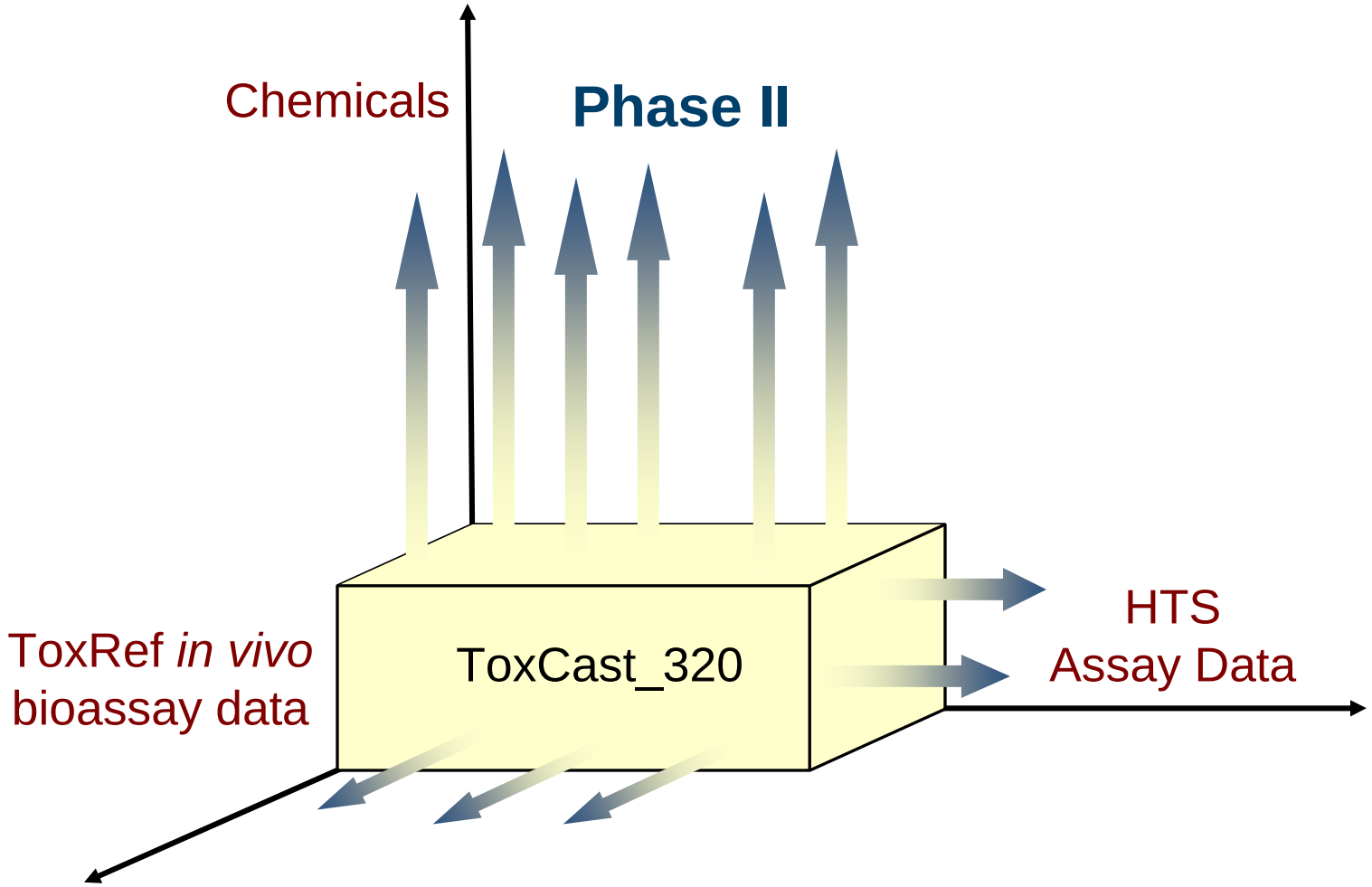
Test \ Truth	+	-
+	TP	FP
-	FN	TN

Pesticide MOA as Endpoint

MOA	Chemicals	Positives	Sensitivity	Specificity	PPV	NPV	Accuracy
thiocarbamate herbicides	303	6	1.00	0.99	0.70	1.00	0.99
dinitroaniline herbicides	303	7	1.00	0.98	0.61	1.00	0.99
Sodium channel modulators	303	11	0.90	0.97	0.51	1.00	0.93
pyrethroid ester insecticides	303	10	0.65	0.98	0.62	0.99	0.81
conazole fungicides	303	13	0.65	0.97	0.52	0.99	0.81
pyridine herbicides	303	6	0.60	0.99	0.67	0.99	0.79
Sodium channel modulators	303	11	0.60	0.98	0.53	0.99	0.79
conazole fungicides	303	13	0.50	0.98	0.53	0.98	0.74
Acetylcholine esterase inhibitors	303	27	0.50	0.97	0.66	0.96	0.74
Acetylcholine esterase inhibitors	303	27	0.52	0.95	0.57	0.96	0.73
pyrethroid ester insecticides	303	10	0.50	0.95	0.32	0.98	0.73
organothiophosphate acaricides	303	9	0.00	1.00	0.00	0.98	0.50
organothiophosphate acaricides	303	9	0.00	1.00	0.00	0.98	0.50
pyridine herbicides	303	6	0.00	1.00	0.00	0.98	0.50
thiocarbamate herbicides	303	6	0.00	1.00	0.00	0.98	0.50
dinitroaniline herbicides	303	7	0.00	0.99	0.00	0.98	0.49

Input variables: NovaScreen, Attagene, Bioseek and physical chemical properties

Beyond the Proof of Concept



Moving Forward

- Completion of Data Acquisition and Data Mining for Phase I
- Publication and Public Release of all Data
- OECD Molecular Screening Initiative
- Data Summit, Fall 2008
- Enlargement
  - Assay partners testing ToxCast_320
  - Analysis partners of Phase I data
  - Resource partners could help expand Phase II
- Related Talks
  - **David Dix**; Use of Genomics Data at the U.S. EPA for Predictive and Mechanistic Toxicology (Abstract #596), **Monday March 17, 3:45pm**, Room 611
  - **Keith Houck**; EPA's ToxCast Program for Predicting Hazard and Prioritizing Toxicity Testing of Environmental Chemicals, **Wednesday March 19, 2:39pm**, Room 6A.